Review paper

Skin surface lipids and their measurements

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Abstract

The lipid coat is the most external protective layer for the skin and hair. Improper functioning of sebaceous glands results in excessive oiliness and dryness of the skin as it is sebocytes that are the main source of lipid substances of sebum, secretion of the sebaceous glands, which is a mixture of lipids such as glycerides, free fatty acids, squalene, wax esters, cholesterol esters and cholesterol. In the past, a lot of methods were used to evaluate the skin lipids and measure their level. At present, a sebumeter and other measuring devices which objectively help to assess the level of skin oiliness are gaining more and more interest. The aim of the study is to present the current knowledge of the skin lipid coat and methods of measuring it.

Key words: skin lipids, sebocytes, sebumeter, measuring sebum secretion.

Lipid coat of the skin

On the surface of the corneal layer there is a skin lipid coat, which is a mixture of sebum secreted by sebaceous glands and epidermal lipids synthesized by keratinocytes. The mixture of these substances mixed with the secretion of sweat glands makes up water in oil (W/O) emulsion, called a hydrolipid coat. It acts as a barrier and regulates processes of absorption and skin penetration of substances soluble in water and fats [1, 2]. Precursors of epidermal lipids are formed in lamellar granules, so called Odland bodies of the granular layer which have secretion properties. Polar lipids (soluble) which are produced by the Odland bodies and catabolic enzymes in the Golgi apparatus are released or transported along canaliculi to the space between the granular and corneal layers. The released material is enzymatically transformed into nonpolar compounds, i.e. insoluble in water, to which hydrolytic enzymes contribute. The disks (contents of lamellar granules) get to the extracellular space, remain in rows on the surface of corneocytes and then fuse. By accumulating in the insoluble lipids of the corneal layer, they create bilamellar structures, so called lamellae (*intercellular lamellae*), which serve as a structural part of a protective barrier of epidermis [3]. In the primary disks there are fatty acids, phospholipids, cholesterol and glucosylceramides, which after exocytosis of lamellar granules are hydrolyzed to ceramides. Final concentrations of lipids in the corneal layer of epidermis are as follows: ceramides – 40%, cholesterol – 25%, fatty acids – 20% and 2-3% for the following: triglycerides, free sphingosine, cholesterol sulphate and cholesterol esters [4].

Sebaceous glands

Sebaceous glands (*glandulae sebaceae*) are the main source of lipids covering the surface of the skin. As for the shape, they are alveolar glands and as for the mechanism of secretion, they are holocrine glands, which results in converting whole cells into a secretion. The cells are then reproduced by the reproductive layer [5]. Sebaceous glands are scattered all over the body except for hands, soles and the dorsum of the feet [6]. The greatest number of the glands is on the face (T-zone), back, chest and it may range between 400 and 900 per square cm [7]. The number of active sebaceous glands and the amount of sebum is different in different people but the shape and distribution in a human body seem to remain the same; however, their size changes – it grows with age [8, 9]. The cells of sebaceous glands, called sebocytes, are modified keratinocytes. The life cycle of sebocytes starts on the circumference of sebaceous glands in their basal layer, where circumferential cells divide and make daughter cells move towards the centre of the gland. While moving, the cells of sebaceous glands differentiate by gathering the lipid drop and increasing the volume. Their secretion appears when adult sebocytes, rich in lipids, atrophy. While disintegrating they release the contents into the duct or directly to the skin surface [5, 10]. The process of secreting sebum on the skin

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surface is induced with a contraction of the smooth muscle, arrectors of hair by impulses of the sympathetic system and is a continuous process. The intensity of the sebaceous glands activity is directly proportional to their size. The activity of the glands depends on hormones. Secretion of sebum is regulated by sex hormones (androgens, estrogens), hormones of the adrenal cortex (corticosteroids) and others [11]. The excessive sebum secretion is caused by:

- dihydrotestosterone DHT – testosterone metabolite, which is formed with the use of 5α-reductase type I [5, 12],
- progesterone – inhibitor of 5α-reductase, the excess of this hormone stimulates the division of sebocytes and increases seborrhea [13],
- prolactin – increases the activity of sebaceous glands during pregnancy and in the second half of the menstrual cycle,
- hormones of the adrenal cortex – cortisol and adrenal androgens,
- growth hormone (GH) [6, 14-16],
- insulin [14, 15, 17],
- adrenaline,
- melanocortins, which include the hormone stimulating melanocytes (α-MSH) and the adrenocorticotropic hormone (ACTH) [5, 18-20],
- thyroid hormones [14, 21-23].

The hormones which reduce the sebum secretion are estrogens [6, 13, 24].

Sebocyte characteristics

One of the most important functions of sebocytes is synthesis of lipids, which, while crystallizing, create a large lipid coat – one of the most vital factors that enables to keep the proper homeostasis in the skin. Ceramides are the main lipids of the epidermis. They constitute a resistant barrier which prevents hydrophilic substances from penetrating the skin as they do not get through lipophilic fragments of the ceramides. The barrier also disallows lipophilic substances to penetrate the skin as they are stopped on the polar groups of ceramides [3]. The sebum of the sebaceous glands transports vitamin E [6, 10, 25, 26] and coenzyme Q10 [27] – antioxidants protecting surface lipids of the skin against oxidizing so the sebum prevents the skin from ageing and keeps its protective barrier. The sebum of the sebaceous glands is unique in terms of a high squalene content, unlike epidermal lipids which contain more cholesterol [28]. It allows for differentiating between lipids secreted by sebaceous glands and epidermal lipids. The lipid environment inside the hair follicle and using the base which would be compatible with the secretion improve the activity of the drugs applied to the skin where they are transported through the follicle. Administering the drug which dissolves in the oil phase and using the solvents which react with the sebum facilitate the accumulation of the drug inside the follicle, which makes it easier to treat certain diseases, e.g. acne [29, 30]. Sebocytes were proved to produce various proinflammatory substances [17]. They include:

- IL-8 – chemokine, which plays an important role in pathogenesis of psoriasis, allergic dermatitis, acne and which is responsible for chemotaxis of inflammatory cells (e.g. neutrophils) to the sebaceous glands and initiates an inflammatory process in the course of many skin diseases [5]. It was also stressed that *Propionibacterium acnes* is not responsible for the sequence of reactions connected with the direct release of two proinflammatory cytokines IL-1α/IL-8, which induce comedogenesis and an inflammatory state of the sebaceous glands. It is also known that these bacteria influence the late phase of stimulating secretion of IL-8 [31]. It was proved that secretion of IL-8 can be modulated by α-MSH [18].
- CRH – (corticotrophin-releasing hormone), the hormone which is released from nerve endings and cells, e.g. from sebocytes. The process is activated by proopiomelanocortin (POMC), a hormone which is activated mainly by proinflammatory cytokines. In sebocyte cultures, CRH increases the expression of mRNA for 3β-hydroxysteroid dehydrogenase (3β-HSD), the key enzyme in the pathway of androgen biosynthesis (it transforms dehydroepiandrosterone into testosterone in human sebocytes) [32]. This neuropeptide increases the production of α-MSH (melanotropin) and this in turn decreases the synthesis of IL-8 in the sebocytes activated by IL-1β *in vitro* [5].
- Free fatty acids – e.g. lauric acid (c12;0) and sapienic acid (C16:1Δ6), which have strong antibacterial properties, especially against Gram-positive bacteria, including *Staphylococcus aureus*, *Streptococcus salivarius* and anaerobic *Fusobacterium nucleatum*. Sebocytes have also an ability to produce their own androgens. They are created from the cholesterol side-chain (P-450scc), which is cut off by the cytochrome P-450 enzyme. Next, with the use of cofactors such as adrenodoxin, adrenodoxin reductase, steroidogenic factor 1 and transcription factor there appears pregnenolone, which at the same time is the precursor of estrogens [33].

In human sebocytes there are steroid nuclear receptors for hormones which are activated by peroxisome proliferators (peroxisome proliferator activated receptors – PPARs). The activation of PPAR regulates a lot of genes responsible for lipid metabolism in peroxisomes, mitochondria and microsomes [6]. The research findings show that PPAR-α is responsible for β-oxidation of free fatty acids, which are PPAR ligands and act as regulators of lipogenesis [34-36]. In the sebaceous gland, in the basal layer of differentiated sebocytes, like in epidermal keratinocytes, there are androgen receptors (AR) and 5α-reductase enzyme, which takes part in converting testosterone into dihydrotestosterone – the most active

form stimulating sebum secretion [11, 24, 33]. New research findings prove the presence of differences in the number and distribution of androgen receptors in various parts of the skin (seborrhea area). It applies both to the sebaceous glands and epidermal keratinocytes [2]. In the hair follicles of the sebaceous glands there are the following receptors:

- growth factor (GF) [6, 16],
- thyroid hormone receptors [22],
- estrogens receptors α and β in resting and partially differentiated sebocytes [24],
- vitamin D receptors in resting nuclei of sebocytes [23],
- retinoic acid receptors (RAR) and retinoic X receptors (RXR) [5, 6, 12, 37],
- melanocortin 1 (MC-1R),
- α-MSH receptor, which was also found in SZ95 cell line [5, 18, 19].

Culture of SZ95 sebocytes

It is necessary to make an analysis of factors and mechanisms which regulate the sebum secretion so as to introduce the right therapy in diseases in which there is an excessive production of lipids or shortage of lipids. Therefore, a lot of studies have been conducted to determine the role of sebocytes in the pathogenesis of various dermatoses. The first experiments were made on fragments of human skin incubated *in vitro* [38]; next on animal models [6] till 1999 when Zouboulis *et al*. found an immortalized sebaceous gland cell line [39].

Immortalized sebocyte line was created by transfecting sebaceous gland skin cells with simian virus 40 large T antigen (SV-40) [18]. The researchers received three clones of SZ95 sebocytes which had properties characteristic of sebocytes – SZ95 /K6, SZ95/K7 as well as of keratinocytes – SZ95/K28. SZ95 cells have the following characteristics [5, 39, 40]:

- they still have their properties despite numerous passages (even more than 50),
- they differ in size up to 3.25 times in the proliferation process and the state of confluence,
- they have polygonal shape because of lipid drops in cytoplasm,
- they contain myelinic structures, endoplasmic reticulum and the Golgi apparatus, which indicates lipid synthesis,
- they possess markers typical of differentiating sebocytes – sebaceous gland antigen, milk fat globulin-2, epithelial sialomucin (MAM-6) and epithelial membrane antigen,
- like ordinary sebocytes they manifest the expression of keratins 7, 13, 19, and type 1 5α-reductase [26].

On the basis of the above characteristics, which are the same for human and immortalized sebocytes, it can be said that SZ95 cell model is extremely invaluable in conducting further studies on physiology and pathology of sebaceous glands.

Sebum composition

Sebum is synthesized in the sebaceous glands which belong to follicular and sebaceous system of the skin. The composition and the amount of the secreted sebum is an individual characteristic and depends on the person's age and his/her hormonal system. The human sebum synthesized in the sebaceous gland consists of triglyceride – 57%, wax esters – 25%, squalene – 15%, cholesterol esters – 2% and cholesterol – 1% [41]. It is believed that free fatty acids which are present on the skin surface appear as a result of partial hydrolysis of triglyceride [42]. Main fatty acids of human sebum have the length of 12 to 20 coal molecules with the prevalence of 16 and 18 molecules. They include lauric acid 12:0 and sapienic acid 16:1 Δ6, which have strong antibacterial properties, especially against Gram-positive bacteria [43, 44].

Acid 16:1 Δ6 appears exclusively in a human and is the most common acid which can be found in sebum [6]. It originates from palmitic acid as a result of the activity of enzyme Δ6 desaturase (specific marker for sebaceous glands) and may constitute up to 25% of all saturated fats in bonds of glycerides [10]. Diunsaturated fatty fats which are present in the sebum include sebalenic acid having the structure 18:2 Δ5 and linolenic acid, whose structure is 18:2 Δ9, 12 which is included in epidermal acylceramides. It is supposed that the substrate of sebalenic acid is palmitic acid 16:0, which thanks to Δ6 desaturase converts into sapienic acid. It undergoes further desaturation in 5, 6 position and its chain is extended to the number of 18 coal molecules [6, 7, 10]. Sebalenic acid is the main component of phospholipids which make up the membrane of sebaceous glands. The increase in the level of the acid leads to a more intensive production of sebum and development of acne. The level of linolenic acid is inversely proportional to the sebum secretion. In patients with seborrhea the level of linoleic acid was lower than the level of sebalenic acid, which is connected with the beginning of the process of comedogenesis [45].

Ramificated fatty acid with methyl units containing saturated and iso- and anti-isomethyl ramificated chains as well as various and multiethyl groups were observed. The arrangement of methyl ramifications within saturated fatty acids is characteristic and remains unchangeable in a particular person – is genetically conditioned [44].

Saturated and strongly unsaturated fatty acids with a small number of double bonds are dominant in the group of esters which are present in the sebum [42]. Wax esters contain saturated and monounsaturated fatty acids in the proportion of 40:60. As for the proportion of esters and cholesterol, it is equal to 65:35 [43].

From the percentage values of the sebum content we can conclude that the characteristic property of human sebum is a high proportion of squalene to cholesterol. Besides, squalene appears only in sebum and that is why it is often used as a marker which allows for telling the

difference between sebaceous lipids and epidermal lipids. Thody and Shuster in their research proved the existence of an incomplete system of enzymes or their reduced activity in the sebaceous gland [7]. In fact a sebaceous gland cannot convert squalene into cholesterol because of an incomplete system of enzymes. Thus, it might imply that cholesterol in isolated sebum comes from epidermis rather than from sebaceous glands [6, 7, 10].

Measuring sebum secretion

Measuring sebum secretion is the focus of constant attention of dermatologists, cosmetologists, pharmacists as well as cosmetic producers. We can do it with various methods which were already used in the past. They include:

The method worked out by Strauss and Pochi, where they used cigarette paper to measure sebum secretion [46-48]. They placed the cigarette paper on the cleansed skin of the forehead and fixed it with a bandage for 3 h. Next they extracted lipids from the paper with ethyl ether and made a quantitative analysis of sebum secretion by weighing it or with thin-layer chromatography [42, 49].

The bentonite method, in which they used bentonite gel and round Dacron discs (1.8 mm diameter) or Dacron net to absorb the sebum from the forehead area [49-51]. The discs or a rectangular fragment of the net was placed on the forehead in a thin layer of the bentonite gel after washing the skin in water with soap and dabbing at it with ethanol. Next, they were covered with an extra amount of bentonite. The total time of the experiment was 24 h. The Dacron discs with the adhered bentonite were replaced with new ones every 3 h, the Dacron net – every 7 h. The rectangular net was replaced with two round discs for the last 3 h. In the course of the experiment it was observed that the amount of the sebum absorbed by the disc is constantly decreasing within the first 12 h, which was interpreted as the excessive sebum secretion coming from follicles. After 12 h the pace of the sebum secretion stopped and remained stable, which corresponded to its synthesis in the glands. The lipids obtained were extracted in ethyl ether and then thin-layer chromatography analysis was carried out [50].

The sebutape method which uses polymer white film [28]. The film is used to measure the sebum secretion [48, 52, 53] and evaluate the distribution of skin pores [8, 54]. The film is placed on the forehead for 3 h and then a computer image analysis is conducted. In the places where the sebaceous secretion has been absorbed the film becomes transparent. Thanks to the placing of the film on the black background we can receive the pore patterns which may be qualitatively matched to the images: preadolescence, adolescence, adult or old [55]. Lipids which are examined with this method can be also extracted and then analyzed with thin-layer chromatography [48, 54]. The sebufix method is also common; here, like in the sebutape method, we use a polymer film to measure the activity of the sebaceous glands. The film is next image-analysed with Visioscan VC98 device [52]. The sebufix method is thus very similar to the sebutape method. The only difference is the fact that in the case of the sebutape method, the film is placed on the selected area for 1-3 h and in the other method – for 30 s [53]. Therefore, the sebufix method allows for measuring sebum secretion within a shorter period of time. Another method uses a standardized device – a sebumeter which objectively facilitates measuring sebum from the selected skin areas [53, 56, 57]. The sebumeter can be used to determine the level of sebum on the skin surface or the pace of its secretion [28, 53, 58-61]. It cannot, however, determine the state of the sebaceous glands [62]. To measure the level of skin oiliness we use a parchment film placed in a special box. In order to gather sebum secretion the film is pressed to the skin of the face, hairy skin of the head, hair or other areas of the body. The film with the sebum gathered is subject to a photometric analysis.

The sebumeter is a simple device, easy to operate, which can be used to make an objective classification of dry skin, normal skin or oily skin [56, 63-65] as well as to evaluate 'the biological age' of the skin [66, 67]. Thanks to the parameters of the skin it is possible to observe changes in it which appear with age and at the same time find ways of preventing and treating potential changes. Vijver *et al*. compared the correlation between the subjective and objective evaluation of the skin oiliness. Three hundred and two volunteers were examined. It turned out that the subjective classification of the skin did not correspond to the amount of the secreted sebum [56, 68].

Lee, Huh *et al.* analysed the activity of superficial chemical peelings such as: 30% glycolic acid and Jessner solution [69]. At every application of the preparation, before and after 2 weeks, the level of sebum on the forehead, nose, chin and cheeks was measured with Sebumeter SM 810. The findings showed that the two peelings applied at 2-week intervals did not lead to the decrease in the amount of the secreted sebum [69]. Sebumetric experiments help to choose the best possible drugs or cosmetics for the patient [52, 70, 71-73]. In other research, changes in the skin physiological parameters after cosmetic application were also analyzed. With the use of Corneometer, Tewameter, Sebumeter the researchers measured water content, transepidermal water loss and sebum secretion in various areas of the facial skin, before and after the application of moisturizing preparations and anti-sebum [52, 70, 71].

Other research aimed at comparing the effect of topical medicaments such as; azelaic acid, benzoyl peroxide, adapalene in acne treatment. All these three topically administered drugs brought positive therapeutic effects; there were hardly any adverse effects. Their activity, however, does not seem to be correlated with sebostatic activity [72].

In another experiment in which 400 male and female volunteers participated, the researchers used the sebumeter to evaluate the effectiveness of 20 kinds of shampoo for greasy hair or for hair with a tendency to become greasy. No considerable differences in the results were observed. It was proved, however, that all the products led to the decrease in sebum secretion after they were used 10 times [38].

The sebumeter enables to pre-assess the type of the skin when it is necessary to check whether a certain person is suitable for a particular job, e.g. a person having dry skin cannot work in the chemical industry [74].

Grunewald and Gloor evaluated the positive effect of hand creams as protective preparations and lipid supplements in patients with contact dermatitis after applying them 5 times a week [75]. They noted the skin was less irritated although it had been exposed to detergents, even while doing hand-washing 5 times a day for a week. The researchers could not offer a full protection after application of various creams; they proved however that application of these positively influences the parameters of skin functions and strengthens the epidermal barrier [75].

Oiliness after application of soaps was also evaluated [76]. Skin care with proper soap is an important factor in the prevention of skin diseases and the loss of lipids induced by constant washing [77].

Scientists Messenger and Birch checked whether there is a relation between the days when it is difficult to do hair and the sebum secretion and menstrual cycle [74, 78]. In the experiment conducted on a group of 13 women, over a period of three menstrual cycles, twice a week, with the use of the sebumeter they measured the level of sebum on the skin of the head and forehead. Simultaneously they registered how often the women washed their hair. They did not observe noticeable differences in the sebum level secreted during menstrual cycle or correlation between the average sebum level and the frequency of days when it was difficult to do hair. Such days, however, appeared more often during a period and in women who rarely wash their hair. Taking the results into consideration the researchers concluded that the frequency of days when it was difficult to do hair does not correspond to the level of the secreted sebum; but infrequent washing hair leads to accumulating sebum on the hair, which might make it difficult to have a proper hairdo [78].

With the use of the sebumeter it is possible to analyze sebo-inhibiting properties of certain cosmetics and anti-acne medicaments [73, 79]. Anadolu *et al.* examined the improvement in effectiveness and tolerance to retinoic acid (RA) in the treatment of *acne vulgaris*, in the form of a new formulation known as β-cyclodextrin (β-CD) [80]. The therapy was applied for 3 months. The sebum and moisture levels were measured with Sebumeter and Corneometer. Having analyzed the received results, the researchers stated that in the five-point scale a complex preparation of hydrogel RA/β-CD (0.025%) obtained improvement which was equal to 4; it was 4.1 – on the moisturizing base and 3 for RA (0.05%). The research findings entirely manifested the improvement in effectiveness and tolerance to the new preparation RA/β-CD (0.025%), especially on the moisturizing base, which is an alternative in treatment of *acne vulgaris*.

Sakai, Kikuchi worked on the activity of sebaceous glands in diabetic patients [81]. Thanks to measurements, they concluded that the activity of glands is decreased. In other research they presented a positive influence of a various cosmetic form in treatment of dry skin and possibility of eliminating not nice effects such as: itching, inflammatory conditions in diabetic patients [82]. The sebumetric estimation of the pilosebaceous follicles in women of menopausal period showed how important hormone replacement therapy is [83]. The signs of hormonal ageing of the skin, appendages and mucous membrane are: decreased secretion of sebum, which results in skin dryness, flabbiness, progressive atrophy, melanogenesis disorders, itching, paroxysmal perspiration and erythema, inhibited process of wound healing. The epidermal barrier becomes less stronger because of decreased production of filagrine, lipids and decreased proliferation of keratinocytes. Oral substitutive hormonal therapy improves the state of the skin and its functions. The influence of substitutive hormonal therapy on the lipid coat of the skin depends on the administered dominant hormone. In the combined hormonal therapy progesterone which has stimulating properties makes the number of lipids grow on the skin surface. When only estrogen is administered less sebum is secreted [13, 84]. The time of application of substitutive hormonal therapy in order to minimize the above symptoms should not exceed 5 years because of a potential risk of breast cancer as well as embolic and thrombotic changes. A certain positive solution might be application of phytoestrogens e.g. daidzein, genistein, which are similar to estrogen receptors in the skin and its appendages. The activity of phytoestrogens is weaker, therefore safer [52, 85].

The instrumental method of measuring sebum production might be used to evaluate the effectiveness of various lasers in decreasing the sebum level. 5 sessions were organized, every 3 weeks. Ten people were examined with laser 1065 nm Nd: Yag, Q-Switched. Next the researchers made an assessment and compared the influence of various parameters. Sebumetric quantitative measurements and image analysis were carried out before and after the therapy. It turned out that ND: YAG is an effective method of decreasing the size of pores and sebum level [27, 86].

Summary

Thanks to a growing number of measuring devices available on the market, it is possible to examine the skin

parameters. It allows for revising data described in the professional literature and at the same time for adjusting therapy in various dermatological disorders.

Excessive secretion of sebum in acne vulgaris, seborrhea can be minimized with anti-sebum preparations, properly selected drugs or procedures in which chemical peelings are used and whose sebostatic activity was confirmed with sebumetric analysis. A similar situation refers to senile skin dryness or atopic dermatitis. In this case, the level of sebum secretion can be measured both before and after the application of substances supplementing lipids of the corneal layer of epidermis. It makes it possible to evaluate the activity of various substances e.g. emollients and as a consequence select the most effective preparations [87].

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